Claims

1. A method for detecting an analyte in a sample comprising the steps of
   - providing detection probes being labeled with a first reporter, which detection probes are capable of binding to the analyte,
   - providing a solid support,
   - providing capture probes being bound or capable of binding to the solid support, which capture probes are capable of binding to the analyte, thus concentrating the analyte on the solid support,
   - contacting the sample with the detection probes, the solid support and the capture probes, and
   - detecting the detection probes, wherein
     - the detection of detection probes is conducted in the presence of quenching probes binding to surplus detection probes not being bound to the analyte and thereby quenching at least partially an emission of the first reporter of said surplus detection probes and/or
     - the solid support is labeled with a second reporter different from the first reporter, imaging the sample at an emission wavelength of the second reporter, generating a mask obtained from imaging the sample at the emission wavelength of the second reporter and applying this mask to an image of the sample used for detecting the detection probes.

2. A method for detecting an analyte in a sample comprising the steps of
   - providing detection oligonucleotides being labeled with a first reporter, which detection oligonucleotides are capable of binding to the analyte,
   - providing a solid support,
   - providing capture oligonucleotides being bound or capable of binding to the solid support, which capture oligonucleotides are capable of binding to the analyte, thus concentrating the analyte on the solid support,

- contacting the sample with the detection oligonucleotides, the solid support and the capture oligonucleotides, and
- detecting the detection oligonucleotides, wherein
  - the detection of detection oligonucleotides is conducted in the presence of quenching oligonucleotides hybridizing to surplus detection oligonucleotides not being bound to the analyte and thereby quenching at least partially an emission of the first reporter of said surplus detection oligonucleotides and/or
  - the solid support is labeled with a second reporter different from the first reporter, imaging the sample at an emission wavelength of the second reporter, generating a mask obtained from imaging the sample at the emission wavelength of the second reporter and applying this mask to an image of the sample used for detecting the detection oligonucleotides.

3. The method according to claim 1 or 2 being conducted in a homogeneous format.

4. The method according to at least one of claims 1 to 3 wherein the first and/or second reporter is luminescent, in particular fluorescent.

5. The method according to at least one of claims 1 to 4 wherein the first and/or second reporter is a dye.

6. The method according to at least one of claims 1 to 5 wherein the detection probes, in particular the detection oligonucleotides, are labeled with a first fluorescent dye and/or the solid support is labeled with a second fluorescent dye.

7. The method according to at least one of claims 2 to 6 wherein a hybrid between detection oligonucleotides and analyte has a higher melting temperature than a hybrid between detection oligonucleotides and quenching oligonucleotides.

8. The method according to claim 7 wherein a melting temperature of a hybrid between detection oligonucleotides and analyte is at least 1 °C, more preferably at least 2 °C, even more preferably at least 5 °C and most preferably at least 10 °C higher than a melting temperature of a hybrid between detection oligonucleotides and quenching oligonucleotides under test conditions.

9. The method according to at least one of claims 2 to 8 wherein contacting the sample with the detection oligonucleotides is performed under first hybridization conditions allowing the generation of a stable hybrid between detection oligonucleotides and analyte.

10. The method according to at least one of claims 2 to 9 wherein contacting the sample with the quenching oligonucleotides is performed under second hybridization conditions allowing the generation of a stable hybrid between surplus detection oligonucleotides not being bound to the analyte and quenching oligonucleotides.

11. The method according to claim 10 wherein said second hybridization conditions do not destabilize a hybrid between detection oligonucleotides and analyte formed under said first hybridization conditions.

12. The method according to at least one of claims 1 to 11 wherein the capture probes, in particular the capture oligonucleotides, are covalently bound to the solid support.

13. The method according to at least one of claims 1 to 11 wherein the capture probes, in particular the capture oligonucleotides, are capable of binding to the solid support via affinity interaction.

14. The method according to claim 13 wherein the capture probes / capture oligonucleotides comprise a first affinity unit capable of binding to a second affinity unit attached to the solid support.

15. The method according to claim 14 wherein the first affinity unit is biotin and the second affinity unit is streptavidin or avidin.

16. The method according to at least one of claims 1 to 15 wherein the solid support is a bead, a cell, a pollen, or a plurality thereof.

17. The method according to at least one of claims 1 to 16 wherein the first reporter labeling the detection probes / detection oligonucleotides differs in its excitation wavelength and/or its emission wavelength from the second reporter labeling the solid support.

18. The method according to claim 17 wherein the difference in the excitation wavelength and/or emission wavelength between first and second reporter is at least 10 nm, preferably at least 20 nm, even more preferably at least 50 nm and most preferably at least 100 nm.

19. The method according to at least one of claims 2 to 17 wherein the detection oligonucleotides comprise a linker sequence, linking the sequence of detection oligonucleotide complementary to the analyte with the first reporter.

20. The method according to at least one of claims 2 to 19 wherein the capture oligonucleotides comprise a linker sequence, linking the sequence of the capture oligonucleotide complementary to the analyte with the affinity unit or the solid support.

21. The method according to at least one of claims 1 to 20 wherein at least two different analytes are being detected by providing at least two different sets of detection probes/detection oligonucleotides and at least two different sets of capture probes/capture oligonucleotides.

22. The method according to claim 21 wherein the different sets of detection probes/detection oligonucleotides are being labeled with different reporters.

23. The method according to claim 22 wherein the reporters of one set are identical, have the same excitation wavelength and/or the same emission wavelength.

24. The method according to at least one of claims 1 to 23 wherein the image recorded at the emission wavelength of the second reporter is recorded simultaneously with the image used for detecting the detection probes/detection oligonucleotides.

25. The method according to claim 24 wherein the image recorded at the emission wavelength of the second reporter is corrected such that it spatially matches with the image used for detecting the detection probes/detection oligonucleotides, or vice versa.

26. The method according to at least one of claims 1 to 25 wherein the quenching probes/quenching oligonucleotides have a quenching unit, said quenching unit preferably being a dye.

27. The method according to at least one of claims 1 to 26 wherein the first reporter is a donor of a Förster resonance energy transfer (FRET) donor-acceptor-pair and the quenching unit is an acceptor of said donor-acceptor-pair.

28. The method according to claim 26 wherein the quenching unit is a dark quencher which quenches at least partially the emission of the first reporter by dissipating an energy of an excited state of the first reporter into the environment.

29. The method according to at least one of claims 1 to 28 comprising the step of quantifying the analyte.

30. The method according to claim 29 wherein the quantification is performed by determining an amount of detection probes/detection oligonucleotides bound to the analyte.

31. The method according to claim 30 wherein the amount of detection probes/detection oligonucleotides bound to the analyte is expressed as the emission intensity emitted by the first reporter.

32. The method according to claim 30 or 31 comprising the step of determining an intensity of a background emission in the vicinity of the solid support and considering such intensity when determining the amount of detection probes/detection oligonucleotides.

33. The method according to at least one of claims 1 to 32 wherein the image of the sample used for detecting the detection probes/detection oligonucleotides is acquired at the emission wavelength of the first reporter.

34. The method according to any one of the preceding claims wherein the detection probes are aptameres, oligonucleotides, or antibodies.

35. The method according to any one of the preceding claims wherein the analyte is a protein or a nucleic acid.

36. The method according to any one of the preceding claims wherein the sample is a cell lysate, in particular a crude cell lysate, or an *in vitro* prepared sample.

37. The method according to claim 21 wherein the capture probes/capture oligonucleotides of different sets are attached or capable of binding to different solid supports.

38. The method according to claim 37 wherein the solid supports differ in the affinity units attached thereto, which affinity units interact with affinity units of the capture probes/capture oligonucleotides.

39. The method according to any of the preceding claims comprising adding a potentially pharmaceutically active substance or a known drug to a cellular sample and analyzing whether such substance induces, inhibits or otherwise modulates the generation of the analyte.

40. Use of the method according to any of the preceding claims in screening for potentially pharmaceutically active substances, in diagnostics, or in determining any potential side effects of drugs.